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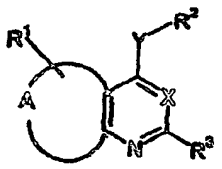
(54) Title: CONDENSED PYRIDINES AND PYRIMIDINES AND THEIR USE AS ALK-5 RECEPTOR LIGANDS



(I)

(57) Abstract: Therapeutically active substituted quinoline and quinazoline compounds derivatives of formula (I) wherein X is N or CH, Y is NH, N (alkyl) or NH-$CH_2$, and $R^2$ and $R^3$ are specified heterorings, the use thereof in therapy, particularly in the treatment or prophylaxis of disorders characterised by overexpression of transforming growth factor β (TGF-β), and pharmaceutical compositions for use in such therapy are disclosed.

# CONDENSED PYRIDINES AND PYRIMIDINES AND THEIR USE AS ALK-5 RECEPTOR LIGANDS

This invention relates to substituted quinoline, quinazoline, thieno[3,2-d]pyrimidine and thieno[2,3-d]pyrimidine compounds which are inhibitors of the transforming growth factor, ("TGF")-ß signaling pathway, in particular, the phosphorylation of smad2 or smad3 by the TGF-β type I or activin-like kinase ("ALK")-5 receptor, to pharmaceutical compositions containing them, methods for their preparation and their use in medicine, specifically in the treatment and prevention of a disease state mediated by this pathway.

TGF-β1 is the prototypic member of a family of cytokines including the TGF-βs, activins, inhibins, bone morphogenetic proteins and Müllerian-inhibiting substance, that signal through a family of single transmembrane serine/threonine kinase receptors. These receptors can be divided into two classes, the type I or activin like kinase (ALK) receptors and type II receptors. The ALK receptors are distinguished from the type II receptors in that the ALK receptors (a) lack the serine/threonine rich intracellular tail, (b) possess serine/threonine kinase domains that are very homologous between type I receptors, and (c) share a common sequence motif called the GS domain, consisting of a region rich in glycine and serine residues. The GS domain is at the amino terminal end of the intracellular kinase domain and is critical for activation by the type II receptor. Several studies have shown that TGF-β signaling requires both the ALK and type II receptors. Specifically, the type II receptor phosphorylates the GS domain of the type I receptor for TGF-β, ALK5, in the presence of TGF-β. The ALK5, in turn, phosphorylates the cytoplasmic proteins smad2 and smad3 at two carboxy terminal serines. The phosphorylated smad proteins translocate into the nucleus and activate genes that contribute to the production of extracellular matrix. Therefore, preferred compounds of this invention are selective in that they inhibit the type I receptor and thus matrix production.

Activation of the TGF-β1 axis and expansion of extracellular matrix are early and persistent contributors to the development and progression of chronic renal disease and vascular disease. Border W.A., *et al, N. Engl. J. Med.*, 1994; 331(19), 1286-92. Further, TGF-β1 plays a role in the formation of fibronectin and plasminogen activator inhibitor-1, components of sclerotic deposits, through the action of smad3

phosphorylation by the TGF-β1 receptor ALK5. Zhang Y., *et al*, *Nature*, 1998; 394(6696), 909-13; Usui T., *et al*, *Invest. Ophthalmol. Vis. Sci.*, 1998; 39(11), 1981-9.

Progressive fibrosis in the kidney and cardiovascular system is a major cause of suffering and death and an important contributor to the cost of health care. TGF-β1 has been implicated in many renal fibrotic disorders. Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. TGF-β1 is elevated in acute and chronic glomerulonephritis Yoshioka K., *et al*, *Lab. Invest.*, 1993; 68(2), 154-63, diabetic nephropathy Yamamoto, T., *et al*, 1993, *PNAS* 90, 1814-1818., allograft rejection, HIV nephropathy and angiotensin-induced nephropathy Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. In these diseases the levels of TGF-β1 expression coincide with the production of extracellular matrix. Three lines of evidence suggest a causal relationship between TGF-β1 and the production of matrix. First, normal glomeruli, mesangial cells and non-renal cells can be induced to produce extracellular-matrix protein and inhibit protease activity by exogenous TGF-β1 in vitro. Second, neutralizing anti-bodies against TGF-β1 can prevent the accumulation of extracellular matrix in nephritic rats. Third, TGF-β1 transgenic mice or in vivo transfection of the TGF-β1 gene into normal rat kidneys resulted in the rapid development of glomerulosclerosis. Kopp J.B., *et al*, *Lab. Invest.*, 1996; 74(6), 991-1003. Thus, inhibition of TGF-β1 activity is indicated as a therapeutic intervention in chronic renal disease.

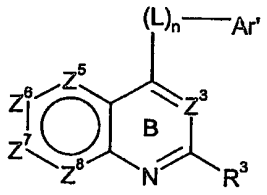
TGF-β1 and its receptors are increased in injured blood vessels and are indicated in neointima formation following balloon angioplasty Saltis J., *et al*, *Clin. Exp. Pharmacol. Physiol.*, 1996; 23(3), 193-200. In addition TGF-β1 is a potent stimulator of smooth muscle cell ("SMC") migration in vitro and migration of SMC in the arterial wall is a contributing factor in the pathogenesis of atherosclerosis and restenosis. Moreover, in multivariate analysis of the endothelial cell products against total cholesterol, TGF-β receptor ALK5 correlated with total cholesterol ($P < 0.001$) Blann A.D., *et al*, *Atherosclerosis*, 1996; 120(1-2), 221-6. Furthermore, SMC derived from human atherosclerotic lesions have an increased ALK5/TGF-β type II receptor ratio. Because TGF-β1 is over-expressed in fibroproliferative vascular lesions, receptor-variant cells would be allowed to grow in a slow, but uncontrolled fashion, while overproducing extracellular matrix components McCaffrey T.A., *et al*, Jr., *J. Clin. Invest.*, 1995; 96(6), 2667-75. TGF-β1 was immunolocalized to non-foamy

macrophages in atherosclerotic lesions where active matrix synthesis occurs, suggesting that non-foamy macrophages may participate in modulating matrix gene expression in atherosclerotic remodeling via a TGF-β-dependent mechanism. Therefore, inhibiting the action of TGF-β1 on ALK5 is also indicated in atherosclerosis and restenosis.

TGF-β is also indicated in wound repair. Neutralizing antibodies to TGF-β1 have been used in a number of models to illustrate that inhibition of TGF-β1 signaling is beneficial in restoring function after injury by limiting excessive scar formation during the healing process. For example, neutralizing antibodies to TGF-β1 and TGF-β2 reduced scar formation and improved the cytoarchitecture of the neodermis by reducing the number of monocytes and macrophages as well as decreasing dermal fibronectin and collagen deposition in rats Shah M., *J. Cell. Sci.*, 1995, 108, 985-1002. Moreover, TGF-β antibodies also improve healing of corneal wounds in rabbits Moller-Pedersen T., *Curr. Eye Res.*, 1998, 17, 736-747, and accelerate wound healing of gastric ulcers in the rat, Ernst H., *Gut*, 1996, 39, 172-175. These data strongly suggest that limiting the activity of TGF-β would be beneficial in many tissues and suggest that any disease with chronic elevation of TGF-β would benefit by inhibiting smad2 and smad3 signaling pathways.

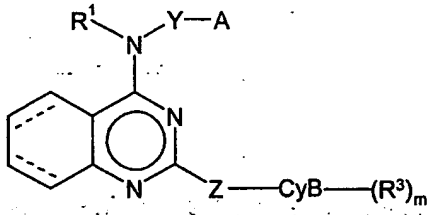
TGF-β is also implicated in peritoneal adhesions Saed G.M., *et al*, *Wound Repair Regeneration*, 1999 Nov-Dec, 7(6), 504-510. Therefore, inhibitors of ALK5 would be beneficial in preventing peritoneal and sub-dermal fibrotic adhesions following surgical procedures.

US 6,476,031 (Scios Inc) discloses methods of inhibiting p38-α activity and/or TGF-β activity using compounds of the formula below :



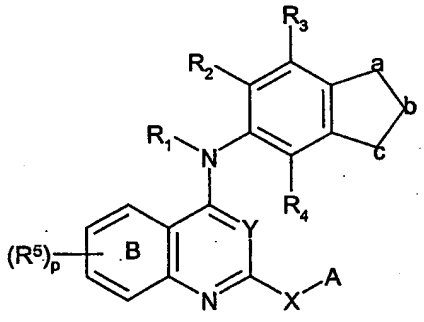

the compounds are stated as being useful in the treatment of conditions including inflammation, proliferative diseases, and certain cardiovascular disorders.

US 5,439,895 (Ono Pharmaceutical Co. Ltd) discloses quinazoline compounds of formula:



The compounds have an inhibitory effect on cGMP-PDE and TXA2 synthase and are said to be useful in the treatment or prevention of hypertension, heart failure, myocardial infarction, angina, atherosclerosis, cardiac edema, renal insufficiency, nephrotic edema, hepatic edema, asthma, bronchitis, dementia, immunodeficiency and pulmonary hypertension.

WO02/076976 (Bayer Corporation) discloses compounds of formula

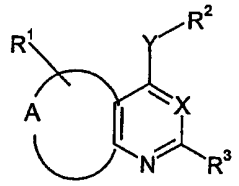


These compounds are said to be Rho-kinase inhibitors and useful in the treatment of hypertension, atherosclerosis, restenosis, cerebral ischemia, cerebral vasospasm, neuronal degeneration, spinal cord injury, cancer of the breast, colon, prostrate, ovaries, brain or lung, thrombotic disorders, asthma, glaucoma, osteoporosis or erectile dysfunction.

Surprisingly, it has now been discovered that a class of substituted quinoline and quinazoline compounds function as potent and selective non-peptide inhibitors of ALK5 kinase and therefore, have utility in the treatment and prevention of various disease states mediated by ALK5 kinase mechanisms, such as chronic renal disease, acute renal disease, wound healing, photoaging of the skin, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein

fibrosis is a major component, including, but not limited to lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis and primary biliary cirrhosis and restenosis.

According to a first aspect, the invention provides a compound of formula (I), a pharmaceutically acceptable salt, solvate or derivative thereof,



(I)

wherein

X is N or CH;

Y is –NH- or –N($C_{1-6}$alkyl)- or -NH-$CH_2$-;

A is a fused 5-, 6- or 7-membered carbocyclic or heterocyclic ring in which one or more of the carbon atoms is optionally replaced by a heteroatom independently selected from N, O and S, and wherein the carbocyclic or heterocyclic ring may be substituted by one or more $R^1$ groups;

$R^1$ is hydrogen, halo, nitro, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, -$CONR^4R^5$, -$O(CH_2)_nNR^4R^5$, -$(CH_2)_nNR^4R^5$ or -$NR^4R^5$, wherein $R^4$ is H or $C_{1-4}$ alkyl, and $R^5$ is $C_{1-4}$ alkyl; or $R^4$ and $R^5$ together with the atom to which they are attached form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S and O;

$R^2$ is pyridinyl, pyrimidinyl, indazolyl, dihydroisoindolyl, benzisoxazolyl, oxazolyl, imidazolyl, oxadiazolyl or thiazolyl, each of which may be substituted by one or more $R^6$ groups;

$R^3$ is pyridin-2-yl, $C_{1-6}$alkylpyridin-2-yl, $C_{1-6}$alkylpyrrol-2-yl or $C_{1-6}$alkylthiazol-2-yl;

$R^6$ is hydrogen, halo, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, -$O(CH_2)_nNR^7R^8$, -$O(CH_2)_n$-$OR^7$, -$NR^7R^8$, -$(CH_2)_nNR^7R^8$, -$CH_2OR^7$ , –$COOR^7$, –$CONR^7R^8$, -$CH_2SO_2NR^7R^8$, -$SO_2NR^7R^8$ or phenyl optionally substituted by one or more groups independently selected from the list: –$OCF_3$, halo, $C_{1-6}$alkoxy, -$CONR^7R^8$, -$SO_2R^7$ , -$O(CH_2)_nNR^7R^8$ , -$(CH_2)_nNR^7R^8$ and -$NR^7R^8$;

$R^7$ and $R^8$, which may be the same or different, are H or $C_{1-6}$alkyl; or $R^7$ and $R^8$, together with the atom to which they are attached, form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S and O; and

n is an integer value from 1 to 6.

The terms "$C_{1-4}$alkyl" or "$C_{1-6}$alkyl" as used herein, whether on their own or as part of a group, refer to a straight or branched chain saturated aliphatic hydrocarbon radical of 1 to 4, and 1 to 6 carbon atoms, respectively, unless the chain length is limited thereto, including, but not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl and hexyl.

The term "alkoxy" as a group or part of a group refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Such alkoxy groups in particular include methoxy, ethoxy, n-propoxy, *iso*-propoxy, n-butoxy, *iso*-butoxy, *sec*-butoxy and *tert*-butoxy.

As used herein, the term "carbocylic" means rings which contain only hydrogen and carbon. The carbocycle may be saturated, unsaturated, or aromatic. Examples of carbocyclic groups include phenyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl and cycloheptyl.

As used herein, the term "heterocyclic" means rings containing one or more heteroatoms selected from nitrogen, sulphur and oxygen atoms. The heterocycle may be aromatic or non-aromatic, i.e. may be saturated, partially or fully unsaturated. Examples of 5-membered groups include thienyl, furanyl, pyrrolidinyl, thiazolyl, oxadiazolyl , oxazolyl, pyrazolyl and imidazolyl. Examples of 6-membered groups include pyridinyl, piperidinyl, pyrimidinyl, piperazinyl and morpholinyl. Certain heterocyclic groups, e.g. thienyl, furanyl, thiazolyl, oxazolyl, pyridinyl and pyrimidinyl, are C-linked to the rest of the molecule. Other heterocyclic groups, e.g pyrrolidinyl, imidazolyl, piperidyl, pyrazolyl, piperazinyl and morpholinyl may be C-linked or N-linked to the rest of the molecule.

The terms "halo" or "halogen" are used interchangeably herein to mean radicals derived from the elements chlorine, fluorine, iodine and bromine.

Preferably X is N.

Preferably Y is –NH-.

Preferably ring A is thiophene, benzene, furan, pyridine, pyrazole or imidazole, each of which may be substituted by one or more $R^1$ groups. More preferably, the ring A is thiophene or benzene, either of which may be substituted by one or more $R^1$ groups. Even more preferably, the ring A is thiophene or benzene either of which may be substituted by one $R^1$ group. Most preferably A is thiophene optionally substituted by one $R^1$ group.

Preferably $R^1$ is hydrogen, halo or $C_{1-6}$alkyl. More preferably $R^1$ is hydrogen or methyl.

Preferably $R^2$ is pyridinyl, pyrimidinyl or indazolyl, each of which may be substituted by one or more $R^6$ groups. More preferably $R^2$ is pyridinyl, halopyridinyl, $C_{1-6}$alkylpyridinyl, pyrimidinyl or indazolyl. Even more preferably $R^2$ is pyridin-4-yl, pyrimidin-4-yl or 1*H*-indazol-5-yl. Still more preferably $R^2$ is pyridin-4-yl.

Preferably, $R^3$ is pyridin-2-yl, 6-methylpyridin-2-yl or 4-methylthiazol-2-yl. More preferably $R^3$ is 6-methylpyridin-2-yl.

Preferably $R^6$ is hydrogen or $C_{1-6}$alkyl. More preferably $R^6$ is hydrogen.

It will be appreciated that the present invention is intended to include compounds having any combination of the preferred groups listed hereinbefore.

A preferred group of compounds of formula (I) is where
X is N;
Y is –NH-;
A is thiophene, benzene, furan, pyridine, pyrazole or imidazole, each of which may be optionally substituted by one of more $R^1$ groups;
$R^1$ is hydrogen, halo or $C_{1-6}$alkyl;
$R^2$ is pyridinyl, pyrimidinyl or indazolyl, each of which may be substituted by one or more $R^6$ groups;

$R^3$ is pyridin-2-yl, 6-methylpyridin-2-yl or 4-methylthiazol-2-yl; and

$R^6$ is hydrogen or $C_{1-6}$alkyl.

Particularly preferred compounds of formula (I) are:

2-(pyridin-2-yl)-4-(pyridin-4-ylamino)-thieno[3,2-d]pyrimidine;

2-(4-Methyl-thiazol-2-yl)-4-(pyridin-4-ylamino)-quinazoline;

2-(6-Methyl-pyridin-2-yl)-4-(pyrimidin-4-ylamino)-thieno[3,2-d]pyrimidine;

2-(6-Methyl-pyridin-2-yl)-4-(1H-indazol-5-ylamino)-quinazoline;

2-(6-Methyl-pyridin-2-yl)-4-(pyridin-4-ylamino)-quinazoline;

2-(6-Methyl-pyridin-2-yl)-4-(pyridin-4-ylamino)-quinoline;

2-(4-Methyl-thiazol-2-yl)-4-(pyridin-4-ylamino)-thieno[3,2-d]pyrimidine;

5-methyl-2-(pyridin-2-yl)-4-(pyridin-4-ylamino)-thieno[2,3-d]pyrimidine;

6-Chloro-2-(6-methyl-pyridin-2-yl)-4-(1H-indazol-5-ylamino)-quinazoline;

2-(6-Methyl-pyridin-2-yl)-4-(pyrimidin-4-ylamino)-quinazoline

and pharmaceutically acceptable salts, solvates and derivatives thereof.

For the avoidance of doubt, unless otherwise indicated, the term substituted means substituted by one or more defined groups. In the case where groups may be selected from a number of alternative groups, the selected groups may be the same or different.

For the avoidance of doubt, the term independently means that where more than one substituent is selected from a number of posssible substituents, those substituents may be the same or different.

As used herein the term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, solvate, ester or amide, or salt or solvate of such ester or amide, of the compound of formula (I), or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) the a compound of formula (I) or an active metabolite or residue thereof, eg, a prodrug. Preferred pharmaceutically acceptable derivatives according to the invention are any pharmaceutically acceptable salts, solvates or prodrugs.

Suitable pharmaceutically acceptable salts of the compounds of formula (I) include acid salts, for example sodium, potassium, calcium, magnesium and tetraalkylammonium and the like, or mono- or di- basic salts with the appropriate acid

for example organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids and the like. Some of the compounds of this invention may be crystallised or recrystallised from solvents such as aqueous and organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Hereinafter, compounds, their pharmaceutically acceptable salts, their solvates and polymorphs, defined in any aspect of the invention (except intermedate compounds in chemical processes) are referred to as "compounds of the invention".

Compounds of the invention may exist in the form of optical isomers, e.g. diastereoisomers and mixtures of isomers in all ratios, e.g. racemic mixtures. The invention includes all such forms, in particular the pure isomeric forms. The different isomeric forms may be separated or resolved one from the other by conventional methods, or any given isomer may be obtained by conventional synthetic methods or by stereospecific or asymmetric syntheses.

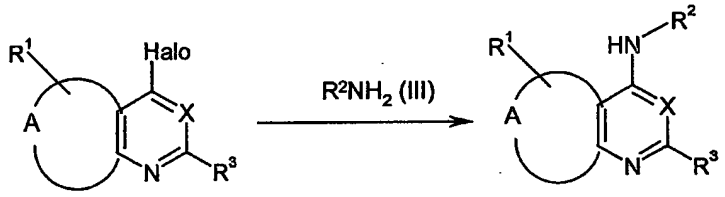
Since the compounds of the invention are intended for use in pharmaceutical compositions it will readily be understood that they are each preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 98% pure (% are on a weight for weight basis). Impure preparations of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions; these less pure preparations of the compounds should contain at least 1%, more suitably at least 5% and preferably from 10 to 59% of a compound of the invention.

Compounds of the invention may be prepared in a variety of ways. In the following reaction schemes and hereafter, unless otherwise stated $R^1$ to $R^3$, X, Y and A are as defined in the first aspect. These processes form further aspects of the invention.

Throughout the specification, general formulae are designated by Roman numerals (I), (II), (III), (IV) etc. Subsets of these general formulae are defined as (Ia), (Ib), (Ic) etc .... (IV), (IVb), (IVc) etc.

Compounds of formula (Ia), i.e. compounds of general formula (I) where Y is NH, may be prepared by reacting compounds of formula (II) where Halo is a halogen atom preferably chloro, with compounds of formula (III) according to reaction scheme 1. Preferred conditions comprise either a) Buchwald coupling using $Pd_2(dba)_3$, BINAP and sodium t-butoxide in toluene at 80°C or b) treatment with NaH in DMF at reflux or DMF at 150°C.

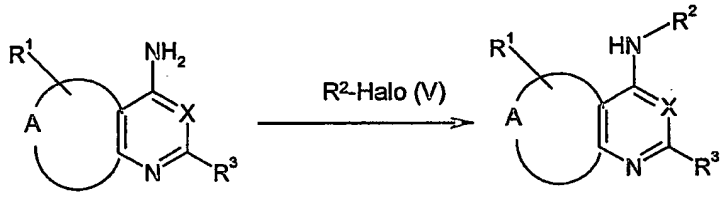
Scheme 1




(II) (Ia)

It will be appreciated that compounds of formula (Ia) may also be prepared according to reaction scheme 2 under analogous reaction conditions to those described for reaction scheme 1.

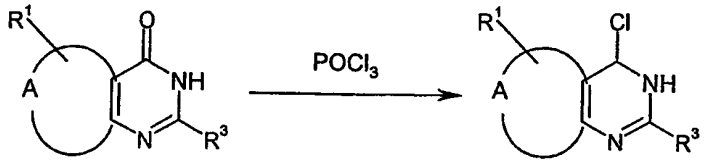
Scheme 2




(IV) (Ia)

Compounds of formula (IIa), i.e. compounds of formula (II) where halo is chloro and Y is NH, may be prepared by treating compounds of formula (VI) with $POCl_3$ in toluene heated under reflux according to reaction scheme 3.

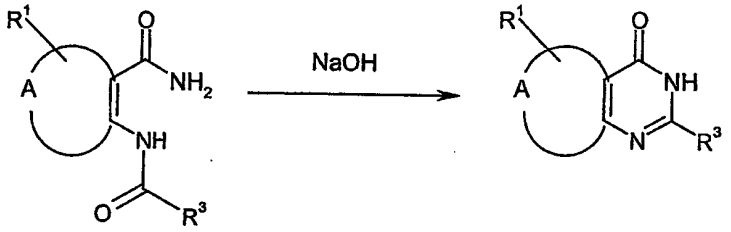
Scheme 3




(VI) (IIa)

Compounds of formula (VI) may be prepared according to reaction scheme 4 from compounds of formula (VII) by reaction with ethanolic sodium hydroxide heated under reflux.

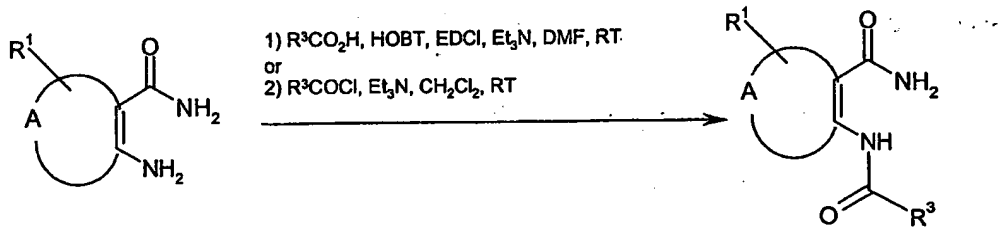
Scheme 4




(VII) (VI)

Compounds of formula (VII) may be prepared according to a variety of methods depending on the nature of $R^1$. Compounds of formula (VII) may be prepared under standard amide coupling conditions (see reaction scheme 5). For example compounds of formula (VIII) may be coupled with carboxylic acid $R^3CO_2H$ by reaction with HOBT, EDCI and triethylamine in dimethylformamide at room temperature. Alternatively compounds of formula (VIII) may be coupled with the corresponding acid chloride $R^3COCl$ by reaction with triethylamine in an inert solvent such as dichloromethane at room temperature.

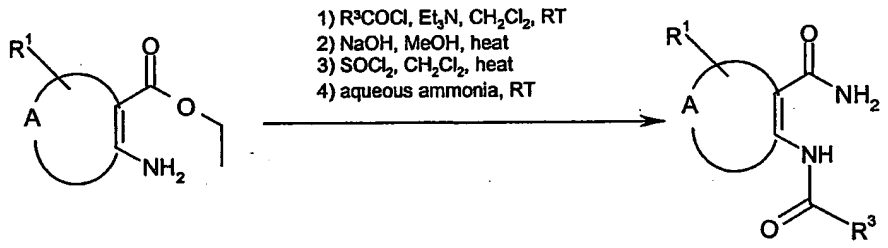
Scheme 5




(VIII) (VII)

Compounds of formula (VII) where $R^1$ is $C_{1-6}$alkoxy or nitro, may be prepared according to reaction scheme 6, by coupling the corresponding ester (IX) with $R^3COCl$ followed by conversion of the resulting ester to amide (VII) using a three step conversion comprising the steps of: a) saponification by heating with methanoilic sodium hydroxide, then b) conversion to the acyl choride by heating with thionyl chloride, then c) treatment with aqueous ammonia at room temperature.

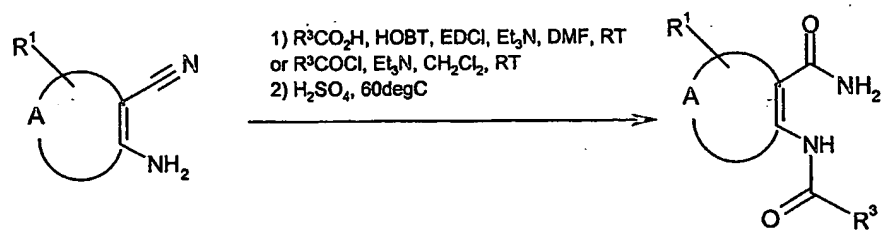
Scheme 6




(IX) (VII)

Alternatively compounds of formula (VII) may be prepared according to reaction scheme 7 involving hydrolysis of compounds of formula (X).

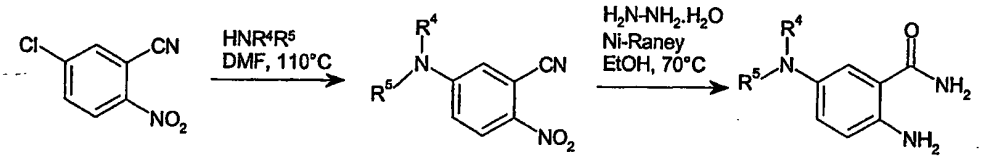
Scheme 7




(X) (VII)

Compounds of formula (VIIIa), i.e. compounds of general formula (VIII) (see scheme 5) where A is phenyl and $R^1$ is $-NR^4R^5$, may be prepared according to reaction scheme 8.

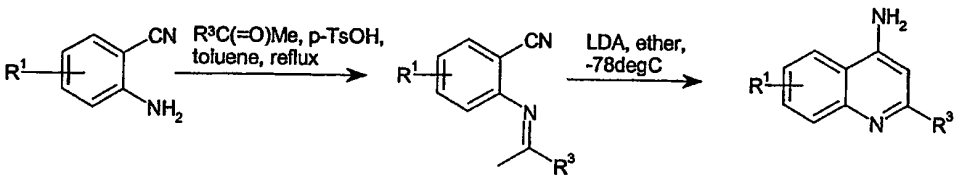
Scheme 8




(VIIIa)

Compounds of formula (IVa), i.e. compounds of formula (IV) (see scheme 2) where A is phenyl and X is CH, may be prepared according to reaction scheme 9. Reaction of compounds of formula (Xa) with $R^3C(=O)Me$ in the presence of para-toluene sulfonic acid under reflux in toluene gave compounds of formula (XI). Reaction of compounds of formula (XI) with lithium diisopropylamine in diethyl ether solvent at low temperature gave compounds of formula (IVa).

Scheme 9




(Xa) (XI) (IVa)

Further details for the preparation of compounds of formula (I) are found in the examples.

The compounds of the invention may be prepared singly or as compound libraries comprising at least 2, for example 5 to 1,000 compounds, and more preferably 10 to 100 compounds. Libraries of compounds of the invention may be prepared by a combinatorial 'split and mix' approach or by multiple parallel synthesis using either solution phase or solid phase chemistry, by procedures known to those skilled in the art. Thus according to a further aspect there is provided a compound library comprising at least 2 compounds of the invention.

Activation of the TGF-β1 axis and expansion of extracellular matrix are early and persistent contributors to the development and progression of chronic renal disease and vascular disease. Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. Further, TGF-β1 plays a role in the formation of fibronectin and plasminogen activator inhibitor-1, components of sclerotic deposits, through the action of smad3 phosphorylation by the TGF-β1 receptor ALK5. Zhang Y., *et al*, *Nature*, 1998; 394(6696), 909-13; Usui T., *et al*, *Invest. Ophthalmol. Vis. Sci.*, 1998; 39(11), 1981-9.

Progressive fibrosis in the kidney and cardiovascular system is a major cause of suffering and death and an important contributor to the cost of health care. TGF-β1 has been implicated in many renal fibrotic disorders. Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. TGF-β1 is elevated in acute and chronic glomerulonephritis Yoshioka K., *et al*, *Lab. Invest.*, 1993; 68(2), 154-63, diabetic nephropathy Yamamoto, T., *et al*, 1993, *PNAS* 90, 1814-1818., allograft rejection, HIV nephropathy and angiotensin-induced nephropathy Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. In these diseases the levels of TGF-β1 expression coincide with the production of extracellular matrix. Three lines of evidence suggest a causal relationship between TGF-β1 and the production of matrix. First, normal glomeruli, mesangial cells and non-renal cells can be induced to produce extracellular-matrix protein and inhibit protease activity by exogenous TGF-β1 in vitro. Second, neutralizing anti-bodies against TGF-β1 can prevent the accumulation of extracellular matrix in nephritic rats. Third, TGF-β1 transgenic mice or in vivo transfection of the TGF-β1 gene into normal rat kidneys resulted in the rapid development of glomerulosclerosis. Kopp J.B., *et al*, *Lab. Invest.*, 1996; 74(6), 991-1003. Thus, inhibition of TGF-β1 activity is indicated as a therapeutic intervention in chronic renal disease.

TGF-β1 and its receptors are increased in injured blood vessels and are indicated in neointima formation following balloon angioplasty Saltis J., *et al*, *Clin. Exp. Pharmacol. Physiol.*, 1996; 23(3), 193-200. In addition TGF-β1 is a potent stimulator of smooth muscle cell ("SMC") migration in vitro and migration of SMC in the arterial wall is a contributing factor in the pathogenesis of atherosclerosis and restenosis. Moreover, in multivariate analysis of the endothelial cell products against total cholesterol, TGF-β receptor ALK5 correlated with total cholesterol ($P < 0.001$) Blann A.D., *et al*, *Atherosclerosis*, 1996; 120(1-2), 221-6. Furthermore, SMC derived from

human atherosclerotic lesions have an increased ALK5/TGF-β type II receptor ratio. Because TGF-β1 is over-expressed in fibroproliferative vascular lesions, receptor-variant cells would be allowed to grow in a slow, but uncontrolled fashion, while overproducing extracellular matrix components McCaffrey T.A., *et al*, Jr., *J. Clin. Invest.*, 1995; 96(6), 2667-75. TGF-β1 was immunolocalized to non-foamy macrophages in atherosclerotic lesions where active matrix synthesis occurs, suggesting that non-foamy macrophages may participate in modulating matrix gene expression in atherosclerotic remodeling via a TGF-β-dependent mechanism. Therefore, inhibiting the action of TGF-β1 on ALK5 is also indicated in atherosclerosis and restenosis.

TGF-β is also indicated in wound repair. Neutralizing antibodies to TGF-β1 have been used in a number of models to illustrate that inhibition of TGF-β1 signaling is beneficial in restoring function after injury by limiting excessive scar formation during the healing process. For example, neutralizing antibodies to TGF-β1 and TGF-β2 reduced scar formation and improved the cytoarchitecture of the neodermis by reducing the number of monocytes and macrophages as well as decreasing dermal fibronectin and collagen deposition in rats Shah M., *J. Cell. Sci.*, 1995, 108, 985-1002. Moreover, TGF-β antibodies also improve healing of corneal wounds in rabbits Moller-Pedersen T., *Curr. Eye Res.*, 1998, 17, 736-747, and accelerate wound healing of gastric ulcers in the rat, Ernst H., *Gut*, 1996, 39, 172-175. These data strongly suggest that limiting the activity of TGF-β would be beneficial in many tissues and suggest that any disease with chronic elevation of TGF-β would benefit by inhibiting smad2 and smad3 signaling pathways.

TGF-β is also implicated in peritoneal adhesions Saed G.M., *et al*, *Wound Repair Regeneration*, 1999 Nov-Dec, 7(6), 504-510. Therefore, inhibitors of ALK5 would be beneficial in preventing peritoneal and sub-dermal fibrotic adhesions following surgical procedures.

Therefore according to a further aspect, the invention provides the use of a compound defined in the first aspect in the preparation of a medicament for treating or preventing a disease or condition mediated by ALK-5 inhibition.

Preferably the disease or condition mediated by ALK-5 inhibition is selected from the list: chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to kidney fibrosis, lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis, primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis, keloids, cancer, abnormal bone function, inflammatory disorders and scarring.

More preferably the disease or condition mediated by ALK-5 inhibition is fibrosis. Preferably kidney fibrosis.

Transforming growth factor-β (TGF-β) is also implicated in photoaging of the skin (see Fisher GJ. Kang SW. Varani J. Bata-Csorgo Z. Wan YS. Data S. Voorhees JJ. , Mechanisms of photoaging and chronological skin aging, *Archives of Dermatology*, 138(11):1462-1470, 2002 Nov. and Schwartz E. Sapadin AN. Kligman LH. "Ultraviolet B radiation increases steady state mRNA (messenger ribonucleic acid) levels for cytokines and integrins in hairless mouse skin- modulation by topical tretinoin", *Archives of Dermatological Research*, 290(3):137-144, 1998 Mar.).

We have found that TGF-β mRNA is elevated after ultraviolet radiation that causes photoaging, suggesting that inhibition of ALK5 is beneficial. This is supported by the results of experiments in animal models described below. Therefore according to an aspect of the present there is provided the use of an ALK5 inhibitor in the preparation of a medicament for treating or preventing photoaging. In addition, according to a further aspect of the present invention there is provided a method for treating or preventing photoaging in a mammal by administering to said mammal an effective amount of an ALK5 inhibitor.

It will be appreciated that references herein to treatment extend to prophylaxis as well as the treatment of established conditions.

Compounds of the present invention may be administered in combination with other therapeutic agents, for example antiviral agents for liver diseases, or in combination with ACE inhibitors or angiotensin II receptor antagonists for kidney diseases.

The compounds of the invention may be administered in conventional dosage forms prepared by combining a compound of the invention with standard pharmaceutical carriers or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical compositions of the invention may be formulated for administration by any route, and include those in a form adapted for oral, topical or parenteral administration to mammals including humans.

The compositions may be formulated for administration by any route. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as

sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilising the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilised powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active material, depending on the method of administration. Where the

compositions comprise dosage units, each unit will preferably contain from 50-500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

It will be recognised by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular mammal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

No toxicological effects are indicated when a compound of the invention is administered in the above-mentioned dosage range.

All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

It will be appreciated that the invention includes the following further aspects. The preferred embodiments described for the first aspect extend these further aspects:

i) a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier or diluent;

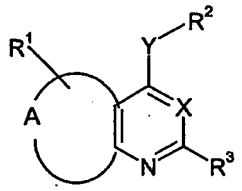
ii) a compound of the invention for use as a medicament;

iii) a method of treatment or prophylaxis of a disorder selected from chronic renal disease, acute renal disease, wound healing, photoaging of the skin, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal

wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to kidney fibrosis, lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis, primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis, keloids, cancer, abnormal bone function, inflammatory disorders and scarring, in mammals, which comprises administration to the mammal in need of such treatment, an effective amount of a compound of the invention; and

iv) a combination of a compound of the invention with an ACE inhibitor or an angiotensin II receptor antagonist.

According to a further aspect, the invention provides a compound of formula (I) or a pharmaceutically acceptable salt, solvate or derivative thereof:



(I)

wherein

X represents N or CH;

Y represents -NH or $-NC_{1-6}$alkyl or $-NH-CH_2-$;

A represents a fused 5-, 6- or 7-membered carbocyclic or heterocyclic ring in which one or more of the carbon atoms is optionally replaced by a heteratom independently selected from N, O and S, and wherein the carbocyclic or heterocyclic ring may be further optionally substituted by one or more $R^1$ groups;

$R^1$ is selected from H, halo, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, $-CH_2CONR^4R^5$, $-O(CH_2)_nNR^4R^5$, $-(CH_2)_nNR^4R^5$, or $-NR^4R^5$, wherein $R^4$ is selected from H or $C_{1-4}$ alkyl, and $R^5$ is $C_{1-4}$ alkyl; or $R^4R^5$ together with the atom to which they are attached form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S or O;

$R^2$ is selected from pyridinyl, pyrimidinyl, indazolyl, dihydroisoindolyl, benzisoxazolyl, oxazolyl, imidazolyl, oxadiazolyl, or thiazolyl, each of which may be further optionally substituted by one or more $R^6$ groups;

$R^3$ is selected from the group comprising pyridinyl, pyrrolyl, thiazolyl, furanyl or thienyl, each of which may be further optionally substituted by one or more $C_{1-6}$alkyl groups;

$R^6$ is selected from H, halo, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, $-O(CH_2)_nNR^7R^8$, $-O(CH_2)_n-OR^7$, $-NR^7R^8$, $-(CH_2)_nNR^7R^8$, $-CH_2OR^7$, $-COOR^7$, $-CONR^7R^8$, $-CH_2SO_2NR^7R^8$, $-SO_2NR^7R^8$, or phenyl optionally substituted by one or more groups selected from $-OCF_3$, halo, $C_{1-6}$alkoxy, $-CONR^7R^8$, $-SO_2R^7$, $-O(CH_2)_nNR^7R^8$, $-(CH_2)_nNR^7R^8$, or $-NR^7R^8$;

$R^7$ and $R^8$ are independently selected from H or $C_{1-6}$alkyl, or $R^7R^8$ together with the atom to which they are attached form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S or O; and

n is an integer value from 1 to 6;

provided that the compound of formula (I) is not:

*N*,2-di-4-pyridinyl-4-quinazolineamine;

*N*-1*H*-indazol-5-yl-2-(2-thienyl)-4-quinazolineamine;

*N*-1*H*-indazol-5-yl-2-(3-thienyl)- 4-quinazolineamine;

5-fluoro-*N*,1*H*-indazoly-5-yl-2-(4-pyridinyl)-4-quinazolineamine;

2-(3-furanyl)-*N*-1*H*-indazol-5-yl-7-methyl-4-quinazolineamine;

*N*-1*H*-indazol-5-yl-7-methyl-2-(3-pyridinyl)-4-quinazolineamine;

*N*-1*H*-indazol-5-yl-7-methyl-2-(4-pyridinyl)- 4-quinazolineamine;

7-chloro-2-(2-furanyl)-*N*-1*H*-indazol-5-yl-4-quinazolineamine; or

7-chloro-*N*-1*H*-indazol-5-yl-2-(3-pyridinyl)-4-quinazolineamine.

The following non-limiting examples illustrate the present invention.

Abbreviations

BINAP - 2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl

$CH_2Cl_2$ - dichloromethane

DMF – dimethylformamide

EDCI – 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride

EtOAc – ethyl acetate

EtOH - ethanol

$Et_3N$ – triethylamine

HOBT - hydroxybenzotriazole

MeOH – methanol

NaH – sodium hydride

$NaHCO_3$ – sodium hydrogen carbonate

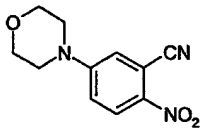
$Pd_2(dba)_3$ – tris(dibenzylideneacetone)dipalladium (0)

$POCl_3$ – phosphorus oxychloride

THF – tetrahydrofuran

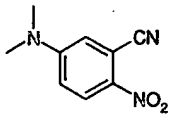
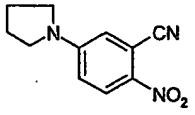
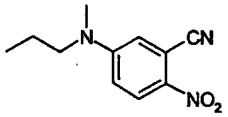
Intermediates

Intermediate 1: 5-(morpholin-4-yl)-2-nitro-benzonitrile

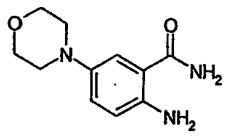


To a solution of 5-chloro-2-nitro-benzonitrile (5.4g, 29.58 mmol) in DMF (50 ml) was added morpholine (5.2 ml, 59.15 mmol) and the mixture was heated at 110°C for 1.5 hours and then concentrated under reduced pressure. The residue was treated with water and the resulting precipitate was filtered and dried. The title compound was obtained as a yellow solid (6.69g, 97.1%); m.p. 209-210°C.

Intermediates 2 to 4 were prepared using methods similar to the preparation of Intermediate 1.

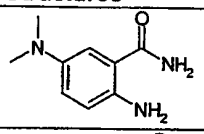
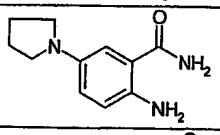
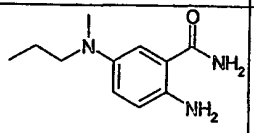
| Int. | Structures | Physical data |
|---|---|---|
| 2 |  | [APCI MS] m/z 192 ($MH^+$) |
| 3 |  | $^1H$ NMR (300MHz, $CDCl_3$, ppm) δ: 8.2 (d, 1H), 6.8 (d, 1H), 6.6 (dd, 1H), 3.4 (m, 4H), 2.1 (m, 4H) |
| 4 |  | [APCI MS] m/z 220 ($MH^+$) |

Intermediate 5: 5-(morpholin-4-yl)-2-amino-benzamide

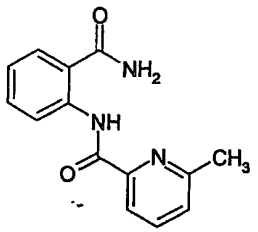


To a solution of intermediate 1 (6.69g, 28.71 mmol) in EtOH (100ml) was added hydrazine monohydrate (2.82 ml, 57.42 mmol) and the mixture was heated at 70°C. Small portions of Raney nickel were added every 5 minutes until no more evolution of gas appeared. After cooling, the catalyst was filtered through a celite pad and washed with EtOH. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on silica gel ($CH_2Cl_2$/MeOH, 95/5 then 9/1). The title compound was obtained as a yellow-green solid (4.5g, 70.92%); [APCI MS] m/z 222 ($MH^+$).

Intermediates 6 to 8 were prepared using methods similar to the preparation of Intermediate 5 from the starting material indicated.

| Int. | Structures | From Int. | Physical data |
|---|---|---|---|
| 6 |  | 2 | [APCI MS] m/z 180 ($MH^+$) |
| 7 |  | 3 | [APCI MS] m/z 206 ($MH^+$) |
| 8 |  | 4 | [APCI MS] m/z 208 ($MH^+$) |

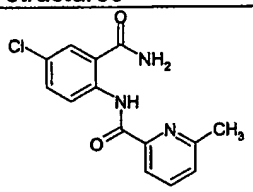
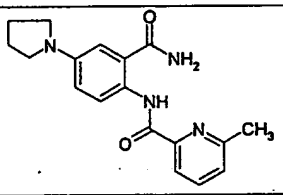
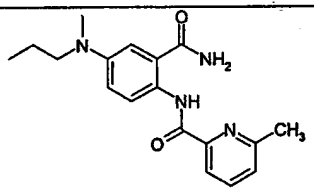
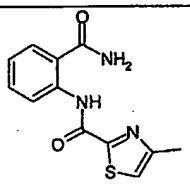
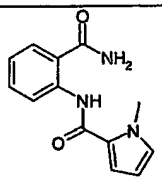
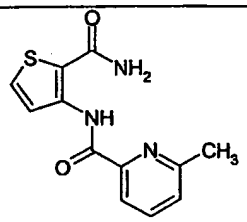
Intermediate 9: 6-Methyl-pyridine-2-carboxylic acid (2-carbamoyl-phenyl)-amide

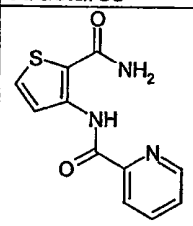
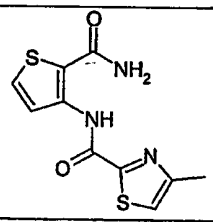
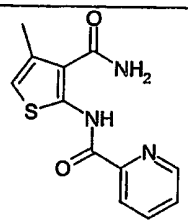
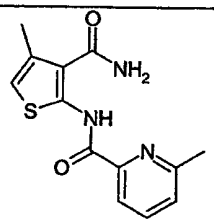
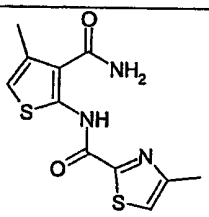
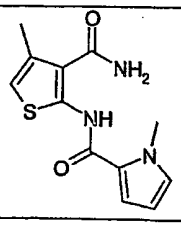


To a solution of 2-amino-benzamide (9g, 66.29 mmol) and 6-methyl-pyridine-2-carboxylic acid (10g, 72.92 mmol) in DMF (180 ml) were added HOBT (9.85g, 72.92

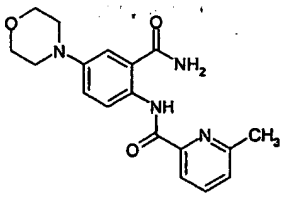
mmol), EDCI (14g, 72.92 mmol) and triethylamine (10.14 ml, 72.92 mmol). The mixture was stirred for 3 h at room temperature. After evaporation, water was added and the precipitate was filtered and washed with water. The title compound was obtained as a white solid (16g, 94.9%); $^1$H NMR (300 MHz, DMSO-d$^6$, ppm) δ : 13.11 (s,1H) ; 8.65 (dd, 1H) ; 8.11 (s,1H) ; 7.83 (s, 1H) ; 7.82 (q, 1H) ; 7.67 (dd, 2H) ; 7.41 (m, 2H) ; 7.05 (td, 1H) ; 2.47 (s, 3H).

Intermediates 10 to 21 were prepared using methods similar to the preparation of Intermediate 9 from the starting material indicated.

| int | structures | From int. | Physical data |
|---|---|---|---|
| 10 |  | 2-amino-5-chloro benzamide and 6-methyl-pyridine-2-carboxylic acid | [APCI MS] 284 ($MH^-$) mp. 264°C |
| 11 |  | 7 | [APCI MS] 325 ($MH^+$) |
| 12 |  | 8 | [APCI MS] m/z 327 $MH^+$ |
| 13 |  | 2-amino-benzamide and 4-methyl-thiazole-2-carboxylic acid | [APCI MS] m/z 262 $MH^+$ |
| 14 |  | 2-amino-benzamide and 1-methyl-pyrrole-2-carboxylic acid | [APCI MS] m/z 244 $MH^+$ |
| 15 |  | 3-amino-thiophene-2-carboxamide and 6-methyl-pyridine-2-carboxylic acid | [APCI MS] m/z 262 $MH^+$ |

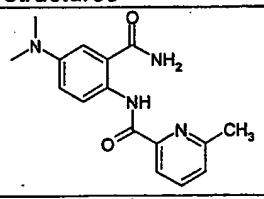
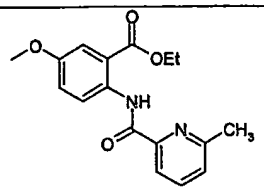
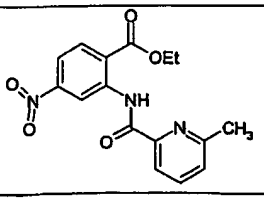
| int | structures | From int. | Physical data |
|---|---|---|---|
| 16 |  | 3-amino-thiophene-2-carboxamide and pyridine-2-carboxylic acid | [APCI MS] m/z 248 $MH^+$ |
| 17 |  | 3-amino-thiophene-2-carboxamide and 4-methyl-thiazole-2-carboxylic acid | [APCI MS] m/z 268 $MH^+$ ; 266 $MH^-$ |
| 18 |  | 2-amino-4-methyl-thiophene-3-carboxamide and pyridine-2-carboxylic acid | [APCI MS] m/z 262 $MH^+$ ; 260 $MH^-$ |
| 19 |  | 2-amino-4-methyl-thiophene-3-carboxamide and 6-methyl-pyridine-2-carboxylic acid | [APCI MS] m/z 274 $MH^-$ |
| 20 |  | 2-amino-4-methyl-thiophene-3-carboxamide and 4-methyl-thiazole-2-carboxylic acid | [APCI MS] m/z 282 $MH^+$ |
| 21 |  | 2-amino-4-methyl-thiophene-3-carboxamide and 1-methyl-pyrrole-2-carboxylic acid | [APCI MS] m/z 264 $MH^+$ |

Intermediate 22 : 6-Methyl-pyridine-2-carboxylic acid -[2-carbamoyl-4-(morpholin-1-yl)-phenyl)-amide

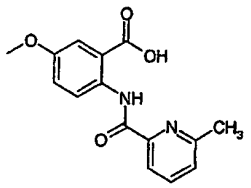


To a solution of 6-methyl-pyridine-2-carboxylic acid (3.35g, 24.43 mmol) in $CH_2Cl_2$ (60 ml) was added dropwise thionyl chloride (30 ml) and the mixture was heated to reflux for 5 hours and then concentrated under reduced pressure. The resulting oil was dissolved in $CH_2Cl_2$ (50 ml) and added dropwise to a solution of intermediate 5 (4.5 g, 20.36 mmol) in $CH_2Cl_2$ (100 ml). Triethylamine (3.4 ml, 24.43 mmol) was then added dropwise and the mixture was stirred at room temperature for 1 hour. The resulting precipitate was filtered, washed with diisopropyl oxide and dried. The title compound was obtained as a pale yellow solid (6.41g, 92.59%); [APCI MS] m/z 341 MH+.

Intermediates 23 to 25 were prepared using methods similar to the preparation of Intermediate 22 from the starting material indicated.

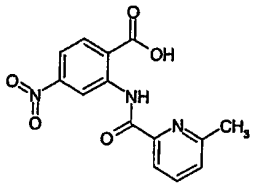
| Int. | structures | From int. | Physical data |
|---|---|---|---|
| 23 |  | 6 | [APCI MS] m/z 299 MH+ |
| 24 |  | Ethyl 2-amino-5-methoxy-benzoate and 6-methyl-pyridine-2-carboxylic acid | $^1$H NMR (300MHz, $CDCl_3$, ppm) δ: 8.85 (d, 1H), 8.05 (d, 1H), 7.7 (t, 1H), 7.55 (sd, 1H), 7.3 (d, 1H), 7.1 (dd, 1H), 4.45 (q, 2H), 3.85 (s, 3H), 2.7 (s, 3H), 1.35 (t, 3H) |
| 25 |  | Ethyl 2-amino-5-nitro-benzoate and 6-methyl-pyridine-2-carboxylic acid | [APCI MS] m/z 330 MH+ |

Intermediate 26 : 6-Methyl-pyridine-2-carboxylic acid -[2-carboxy-4-methoxy-phenyl)-amide



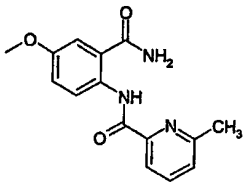
To a solution of intermediate 24 (5 g, 15.92 mmol) in EtOH (30 ml) was added a solution of NaOH 1N (31.8 ml, 31.8 mmol) and the mixture was heated under reflux for 1 hour and then cooled. A solution of HCl 1N (31.8 ml) was added and the resulting precipitate was filtered and dried. The title compound was obtained as a grey solid (3.676g, 81%); [APCI MS] m/z 287 MH+.

Intermediate 27 : 6-Methyl-pyridine-2-carboxylic acid –(2-carboxy-5-nitro-phenyl)-amide



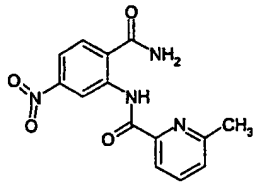
The title compound was prepared from intermediate 25 (15.3g, 46,6 mmol) using an analogous procedure to that described for Intermediate 26; [APCI MS] m/z 302 MH+.

Intermediate 28 : 6-Methyl-pyridine-2-carboxylic acid -[2-carbamoyl-4-methoxy-phenyl]-amide



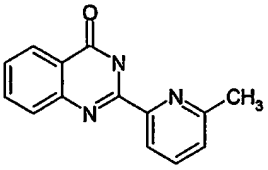
To a solution of intermediate 26 (3.676 g, 12.85 mmol) in $CH_2Cl_2$ (200 ml) was added dropwise thionyl chloride (7.5 ml, 102.8 mmol) and the mixture was heated at 50°C for 3 hours and then concentrated under reduced pressure. The resulting solid was added portionwise to an aqueous solution of ammonia 25% (200 ml) and the mixture was stirred at room temperature for 2 hours. The resulting precipitate was filtered and dried. The title compound was obtained as a grey solid (3.472g, 95%); [APCI MS] m/z 286 MH+.

Intermediate 29 : 6-Methyl-pyridine-2-carboxylic acid -[2-carbamoyl-5-nitro-phenyl]-amide



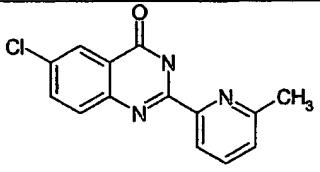
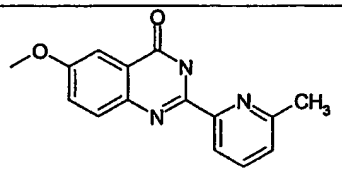
The title compound was prepared from intermediate 27 (17.7g, 58.8 mmol) using an analogous procedure to that described for Intermediate 26 (11.4g, 65%); [APCI MS] m/z 301 MH+.

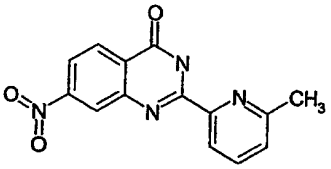
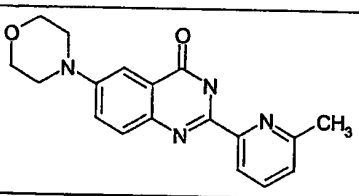
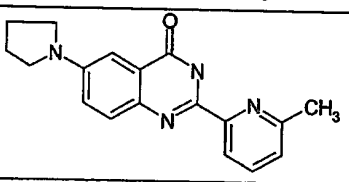
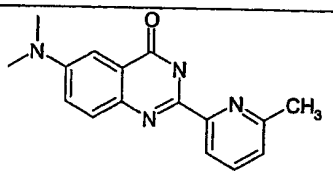
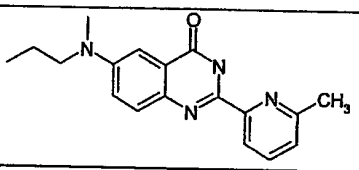
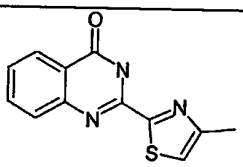
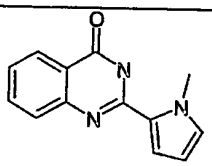
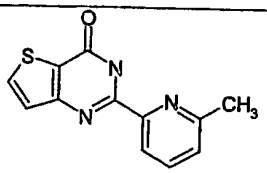
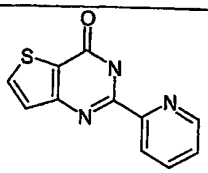
Intermediate 30 : 2-(6-methyl-pyridin-2-yl)-3H-quinazolin-4-one

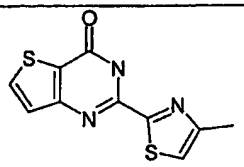
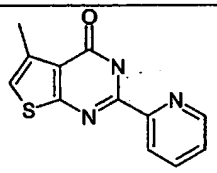
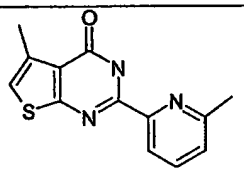
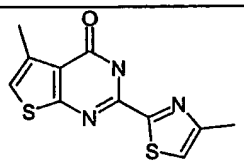
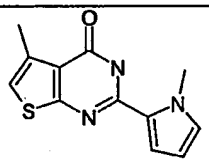


To a solution of intermediate 9 (41.5g, 162.74 mmol) in MeOH (100ml) was added a solution of NaOH 1N (500 ml) and the mixture was refluxed for 3 h. Then the mixture was poured into water and neutralised with a concentrated solution of HCl to give a precipitate which was filtered and washed with water and dried. The title compound was obtained as a white solid (38.8g, 99.63%); [APCI MS] m/z 238 $MH^+$.

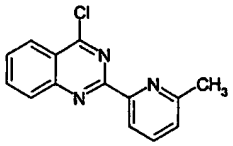
Intermediates 31 to 46 were prepared using methods similar to the preparation of Intermediate 30 from the starting material indicated.

| int. | structures | from Int. | Physical data |
|---|---|---|---|
| 31 |  | 10 | mp. 212°C<br>[APCI MS] m/z 272 $MH^+$ |
| 32 |  | 28 | [APCI MS] m/z 268 $MH^+$ |

| int. | structures | from Int. | Physical data |
|---|---|---|---|
| 33 |  | 29 | [APCI MS] m/z 283 $MH^+$ |
| 34 |  | 22 | [APCI MS] m/z 323 $MH^+$ |
| 35 |  | 11 | [APCI MS] m/z 307 $MH^+$ |
| 36 |  | 23 | [APCI MS] m/z 281 $MH^+$ |
| 37 |  | 12 | [APCI MS] m/z 309 $MH^+$ |
| 38 |  | 13 | [APCI MS] m/z 244 $MH^+$ |
| 39 |  | 14 | [APCI MS] m/z 226 $MH^+$ |
| 40 |  | 15 | [APCI MS] m/z 244 $MH^+$ |
| 41 |  | 16 | [APCI MS] m/z 230 $MH^+$ |

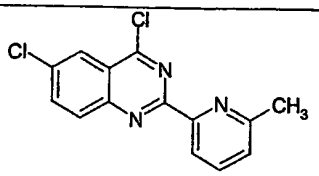
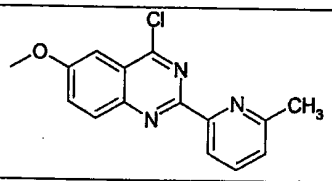
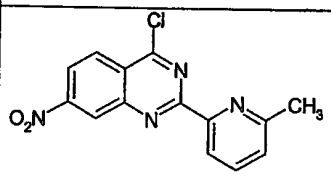
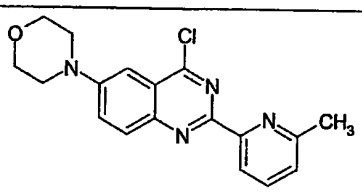
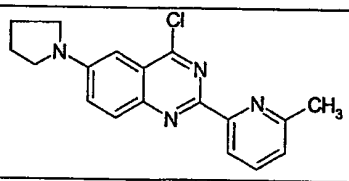
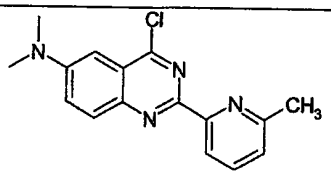
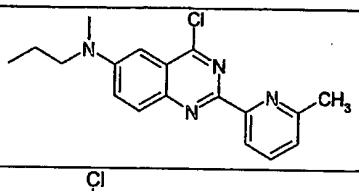
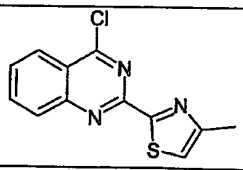
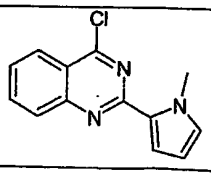
| int. | structures | from Int. | Physical data |
|---|---|---|---|
| 42 |  | 17 | [APCI MS] m/z 250 $MH^+$ |
| 43 |  | 18 | [APCI MS] m/z 244 $MH^+$ |
| 44 |  | 19 | [APCI MS] m/z 258 $MH^+$ |
| 45 |  | 20 | [APCI MS] m/z 264 $MH^+$ |
| 46 |  | 21 | [APCI MS] m/z 246 $MH^+$ |

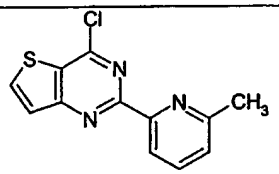
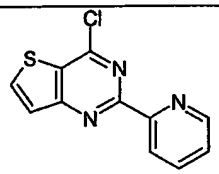
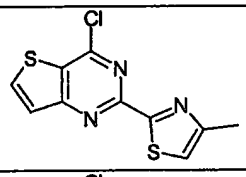
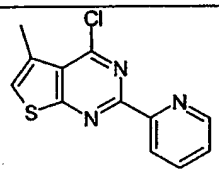
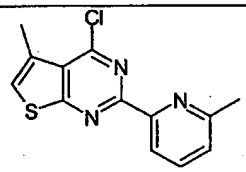
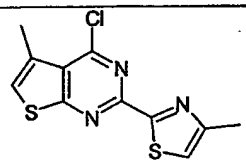
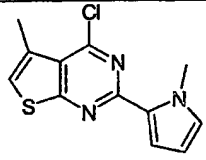
Intermediate 47: 4-Chloro-2-(6-methyl-pyridin-2-yl)-quinazoline



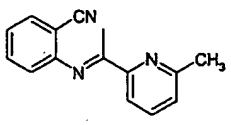
To a suspension of intermediate 30 (31.3g, 132.1 mmol) in toluene (300 ml) was added dropwise $POCl_3$ (100 ml). The resulting mixture was heated at 90°C overnight and evaporated to dryness. The residue was treated with a saturated aqueous solution of $NaHCO_3$ and extracted with $CH_2Cl_2$ . The organic phase was dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the title compound as a cream solid (31.6g, 93.65%); [APCI MS] m/z 256 ($MH^+$).

Intermediates 48 to 63 were prepared using methods similar to the preparation of Intermediate 47 from the starting materials indicated.

| int. | structures | from Int. | Physical data |
|---|---|---|---|
| 48 |  | 31 | [APCI MS] m/z 291 $MH^+$ |
| 49 |  | 32 | [APCI MS] m/z 286 MH+ |
| 50 |  | 33 | [APCI MS] m/z 301 MH+ |
| 51 |  | 34 | [APCI MS] m/z 341 MH+ |
| 52 |  | 35 | [APCI MS] m/z 325 MH+ |
| 53 |  | 36 | [APCI MS] m/z 299 MH+ |
| 54 |  | 37 | [APCI MS] m/z 327 MH+ |
| 55 |  | 38 | [APCI MS] m/z 262 MH+ |
| 56 |  | 39 | [APCI MS] m/z 244 MH+ |

| int. | structures | from Int. | Physical data |
|---|---|---|---|
| 57 |  | 40 | [APCI MS] m/z 262 MH+ |
| 58 |  | 41 | [APCI MS] m/z 248 MH+ |
| 59 |  | 42 | [APCI MS] m/z 268 MH+ |
| 60 |  | 43 | [APCI MS] m/z 262 MH+ |
| 61 |  | 44 | [APCI MS] m/z 276 MH+ |
| 62 |  | 45 | [APCI MS] m/z 282 MH+ |
| 63 |  | 46 | [APCI MS] m/z 264 MH+ |

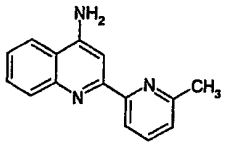
Intermediate 64: 2-[[1-(6-methyl-pyridin-2-yl)ethylidene]amino]benzonitrile



To a solution of 2-aminobenzonitrile (6.11 g, 51mmol) and 2-acety-6-methylpyridine (7g, 51 mmol) in toluene (100 ml) was added paratoluenesulfonic acid (10mg) with azeotropic removal of water. The mixture was heated to reflux overnight. The mixture was poured into water and extracted with toluene. The organic layer was dried over

$Na_2SO_4$ and evaporated under reduced pressure to give the title compound as an oil (8.1g, 67.6%); GCMS m/z 235.

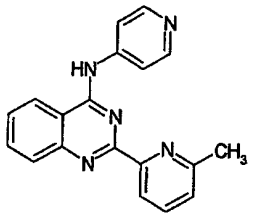
Intermediate 65: 4-Chloro-2-(6-methyl-pyridin-2-yl)-quinazoline



A solution of intermediate 64 (8.1g, 34mmol) in diethyl ether (150mL) was added to a solution of lithium diisopropylamine (1.8M in hexane, 39mL, 69mmol) at –78°C. The reaction mixture was allowed to stir at room temperature for 3 hours and was quenched with water (120mL). The mixture was acidified with HCl (1N) to pH1 and extracted with EtOAc. The aqueous layer was basified with $NaHCO_3$. The organic layer was extracted with EtOAc, washed with water, dried and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with $CH_2Cl_2$/ MeOH 90:10, to give the title compound as a yellow solid (3.6g, 45%); [APCI MS] m/z 236 MH+.

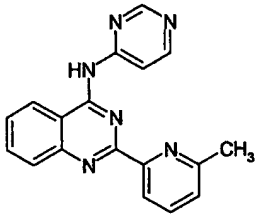
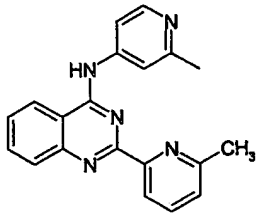
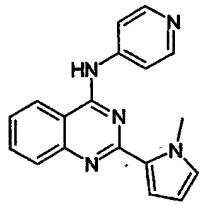
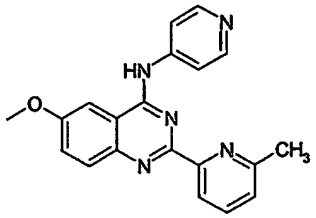
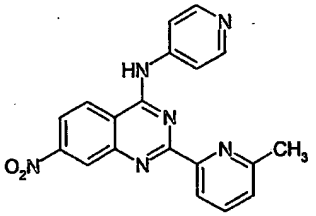
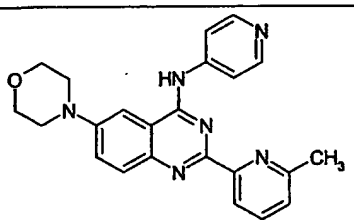
Examples

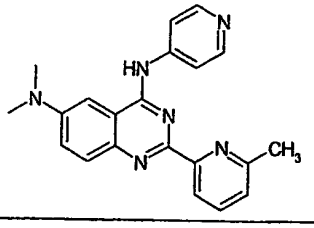
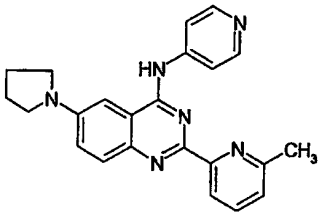
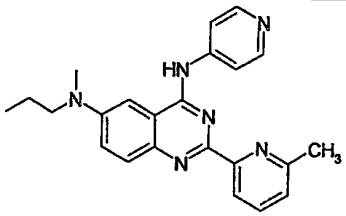
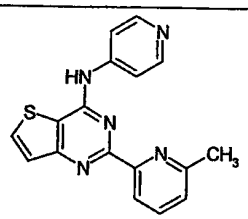
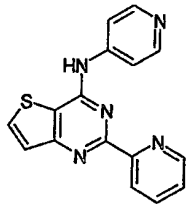
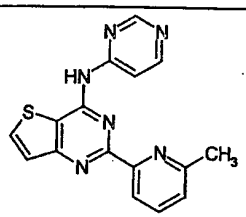
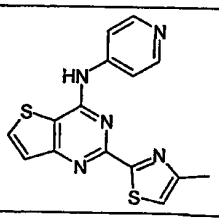
Example1: 2-(6-Methyl-pyridin-2-yl)-4-(pyridin-4-yl-amino)-quinazoline

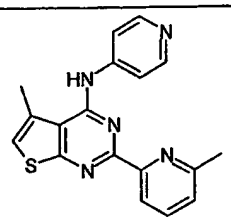
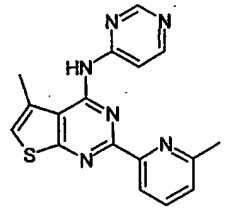
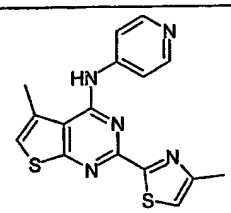
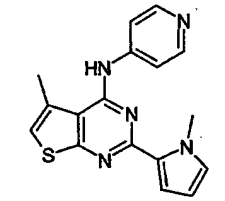
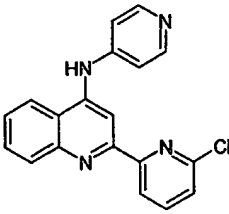


A mixture of intermediate 47 (4g, 15.65 mmol), pyridin-4-yl-amine (2.95g, 31.31 mmol), $Pd_2(dba)_3$ (716 mg, 0.78 mmol), BINAP (1.46g, 2.35 mmol) and sodium *tert*-butoxide (2.46g, 21.92 mmol) in toluene (100 ml) was heated to reflux for 2 hours and then poured into water. The aqueous layer was then extracted 2 times with $CH_2Cl_2$ (300 ml and 100 ml) and the combined organic extracts were washed with water (300 ml), dried over $Na_2SO_4$ and filtered. Evaporation of the solvent under reduced pressure, gave the crude compound which was purified by chromatography on silica gel ($CH_2Cl_2$/MeOH 96/4). After recrystallisation from acetonitrile, the title compound was obtained as a pale yellow crystals (2.06g, 42%); [LC Tof] $C_{19}H_{15}N_5$ ($MH^+$) calculated 314.1406; ($MH^+$) found 314.1400; m.p. gummy at 135-139°C.

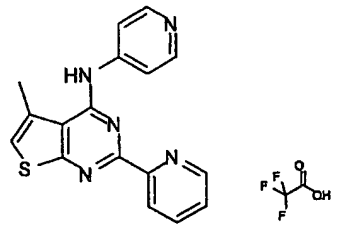
Examples 2 to 19 were prepared using methods similar to the preparation of Example 1 from the starting material indicated.

| Ex. | structures | From int. | Physical data |
|---|---|---|---|
| 2 |  | 47 | [LC Tof] $C_{18}H_{14}N_6$ ($MH^+$) calculated 315.1358; ($MH^+$) found 315.1285 ; m.p. 215°C |
| 3 |  | 47 | [LC Tof] $C_{20}H_{17}N_5$ ($MH^+$) calculated 328.1562; ($MH^+$) found 328.1490 m.p. gummy at 110°C |
| 4 |  | 56 | [LC Tof] $C_{18}H_{15}N_5$ ($MH^+$) calculated 302.1406; ($MH^+$) found 302.1292; m.p. 252°C |
| 5 |  | 49 | [LC Tof] $C_{20}H_{17}N_5O$ ($MH^+$) calculated 344.1511; ($MH^+$) found 344.1491 m.p. 224°C |
| 6 |  | 50 | [LC Tof] $C_{19}H_{14}N_6O_2$ ($MH^+$) calculated 359.1256; ($MH^+$) found 359.1239; m.p. 135°C |
| 7 |  | 51 | [LC Tof] $C_{23}H_{22}N_6O$ ($MH^+$) calculated 399.1933; ($MH^+$) found 399.1927 m.p. 193-195°C |

| Ex. | structures | From int. | Physical data |
|---|---|---|---|
| 8 |  | 53 | [LC Tof] $C_{21}H_{20}N_6$ ($MH^+$) calculated 357.1828; ($MH^+$) found 357.1838 m.p. gummy at 183°C |
| 9 |  | 52 | [APCI MS] m/z 383 MH+; m.p. 182°C |
| 10 |  | 54 | [LC Tof] $C_{23}H_{24}N_6$ ($MH^+$) calculated 386.2219; ($MH^+$) found 386.2185 m.p. 160°C |
| 11 |  | 57 | [LC Tof] $C_{17}H_{13}N_5S$ ($MH^+$) calculated 320.0970; ($MH^+$) found 320.0972 [APCI MS] m/z 320 ($MH^+$) ; 318 (MH-);m.p. gummy at 163°C |
| 12 |  | 58 | [APCI MS] m/z 306 ($MH^+$) ; 304 (MH-); m.p. gummy at 135-140°C |
| 13 |  | 57 | [LC Tof] $C_{16}H_{12}N_6S$ ($MH^+$) calculated 321.0922 ; ($MH^+$) found 321.0899 [APCI MS] m/z 321($MH^+$) ; 319 (MH-); m.p. 218°C |
| 14 |  | 59 | [LC Tof] $C_{15}H_{11}N_5S_2$ ($MH^+$) calculated 326.0534; ($MH^+$) found 326.0527;[APCI MS] m/z 326 ($MH^+$); 324 ($MH^-$); m.p. 236°C |

| Ex. | structures | From int. | Physical data |
|---|---|---|---|
| 15 |  | 61 | [LC Tof] $C_{18}H_{15}N_5S$ ($MH^+$) calculated 334.1126; ($MH^+$) found 334.1136 |
| 16 |  | 61 | [LC Tof] $C_{17}H_{14}N_8S$ ($MH^+$) calculated 335.1000; ($MH^+$) found 335.1079; m.p. gummy at 160°C |
| 17 |  | 62 | [LC Tof] $C_{16}H_{13}N_5S_2$ ($MH^+$) calculated 340.0691; ($MH^+$) found 340.0691 [APCI MS] m/z 340 ($MH^+$); 338($MH^-$)m.p. 240°C |
| 18 |  | 63 | [LC Tof] $C_{17}H_{15}N_5S$ ($MH^+$) calculated 322.1126 ; ($MH^+$) found 322.1126 [APCI MS] m/z 322 ($MH^+$) m.p. 211°C |
| 19 |  | 65 | [LC Tof] $C_{20}H_{16}N_4$ ($MH^+$) calculated 313.1453 ; ($MH^+$) found 313.1477 [APCI MS] m/z 313 ($MH^+$) m.p. 226°C |

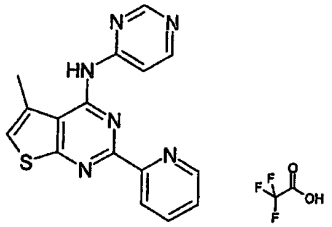
Example 20: 5-methyl-2-(pyridin-2-yl)-4-(pyridin-4-ylamino)-thieno[2,3-*d*]pyrimidine trifluoroacetate



4-Aminopyridine (36 mg, 0.38 mmol) was dissolved in 5 ml of DMF. NaH (60% dispersion in mineral oil, 46 mg, 1.14 mmol) was added and the mixture was stirred under nitrogen for 10 minutes. Intermediate 60 (37 mg, 0.38 mmol) was added, and

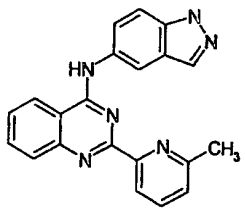
the mixture was stirred at room temperature for 18 hours. The reaction mixture was purified on LC-18 reverse phase silica gel with $H_2O$/ $CH_3CN$ /0.1% TFA (0 to 100% $CH_3CN$ over 40 minutes) to yield the title compound as a white solid (9.3 mg); $^1H$ NMR (400 MHz, DMSO-$d^6$) δ ppm 2.73 (d, *J*=1.10 Hz, 3 H) 7.56 (ddd, *J*=7.37, 4.71, 1.10 Hz, 1 H) 7.65 (d, *J*=1.28 Hz, 1 H) 8.00 (td, *J*=7.74, 1.74 Hz, 1 H) 8.21 (d, *J*=7.32 Hz, 2 H) 8.41 (d, *J*=7.87 Hz, 1 H) 8.67 (d, *J*=7.32 Hz, 2 H) 8.78 (d, *J*=4.03 Hz, 1 H) 10.20 (s, 1 H); MS m/z 320 $(M+H)^+$

Example 21: 5-methyl-2-(pyridin-2-yl)-4-(pyrimidin-4-ylamino)-thieno[2,3-*d*]pyrimidine Trifluoroacetate



4-Aminopyrimidine (36 mg, 0.38 mmol) was dissolved in 5 ml of DMF. NaH (60% dispersion in mineral oil) (46 mg, 1.14 mmol) was added and the mixture was stirred under nitrogen for 10 minutes. Intermediate 60 (37 mg, 0.38 mmol) was added. The mixture was stirred at room temperature for 18 hours. The reaction mixture was purified on LC-18 reverse phase silica gel with $H_2O$ / $CH_3CN$ / 0.1% TFA (0 to 100% $CH_3CN$ over 40 minutes) to yield the title compound as a light yellow solid (64 mg); 1H NMR (400 MHz, DMSO-$d_6$) δ ppm 2.73 (d, *J*=0.73 Hz, 3 H) 7.62 (s, 1 H) 7.71 (ddd, *J*=6.77, 5.13, 0.55 Hz, 1 H) 8.17 (td, *J*=7.83, 1.37 Hz, 1 H) 8.52 (m, *J*=8.06 Hz, 2 H) 8.69 (d, *J*=5.86 Hz, 1 H) 8.85 (d, *J*=4.03 Hz, 1 H) 8.99 (s, 1 H) 9.87 (s, 1 H); MS m/z 321 $(M+H)^+$

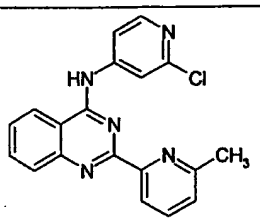
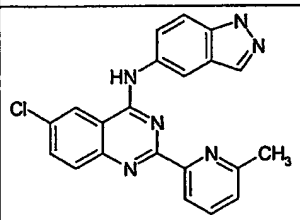
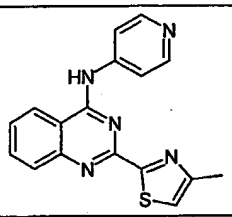
Example 22: 4-(1H-Indazol-5-yl-amino)-2-(6-methyl-pyridin-2-yl)-quinazoline



A solution of intermediate 47 (1.5g, 5.87 mmol), 1H-indazol-5-ylamine (937 mg, 7.04 mmol) and DMF (a few drops) was heated at 140°C for 5 min. Water was added and the resulting precipitate was filtered and dried. After crystallisation from acetonitrile,

the title compound was obtained as crystals (63 mg); [APCI MS] m/z 353 $MH^+$; m.p. 208°C.

Examples 23 to 25 were prepared using methods similar to the preparation of Example 22 from the starting materials indicated.

| Ex. | structures | From int. | Physical data |
|---|---|---|---|
| 23 |  | 47 | [LC Tof] $C_{19}H_{14}ClN_5$ ($MH^+$) calculated 348.1016; ($MH^+$) found 348.1022; m.p. gummy at 135°C |
| 24 |  | 48 | [LC Tof] $C_{21}H_{15}ClN_6$ ($MH^+$) calculated 387.1125; ($MH^+$) found 387.1130; m.p. 195°C |
| 25 |  | 55 | [APCI MS] m/z 320 ($MH^+$); 318 ($MH^-$); m.p. gummy at 150°C |

Biological Assays

The biological activity of the compounds of the invention may be assessed using the following assays:

Assay 1 (Cellular transcriptional assay)

The potential for compounds of the invention to inhibit TGF-β signaling may be demonstrated, for example, using the following *in vitro* assay.

The assay was performed in HepG2 cells stably transfected with the PAI-1 promoter (known to be a strong TGF-β responsive promoter) linked to a luciferase (firefly) reporter gene. The compounds were selected on their ability to inhibit luciferase activity in cells exposed to TGF-β. In addition cells were transfected with a second

luciferase (Renilla) gene which was not driven by a TGF-β responsive promoter and was used as a toxicity control.

(96 well-)microplates were seeded, using a multidrop apparatus, with the stably transfected cell line at a concentration of 35000 cells per well in 200 µl of serum-containing medium. These plates were placed in a cell incubator.
18 to 24 hours later (Day 2), cell-incubation procedure is launched. Cells are incubated with TGF-β and a candidate compound at concentrations in the range 50 nM to 10 µM (final concentration of DMSO 1%). The final concentration of TGF-β (rhTGFβ-1) used in the test was 1 ng/mL. Cells were incubated with a candidate compound 15-30 mins prior to the addition of TGF-β. The final volume of the test reaction was 150 µl. Each well contained only one candidate compound and its effect on the PAI-1 promoter was monitored.

Columns 11 and 12 were employed as controls. Column 11 contained 8 wells in which the cells were incubated in the presence of TGF-β, without a candidate compound. Column 11 was used to determine the 'reference TGF-β induced firefly luciferase value' against which values measured in the test wells (to quantify inhibitory activity) were compared. In wells A12 to D12, cells were grown in medium without TGF-β. The firefly luciferase values obtained from these positions were representive of the 'basal firefly luciferase activity'. In wells E12 to H12, cells were incubated in the presence of TGF-β and 500 µM CPO (Cyclopentenone, Sigma), a cell toxic compound. The toxicity was revealed by decreased firefly and renilla luciferase activities (around 50 % of those obtained in column 11).

12 to 18 hours later (day 3), the luciferase quantification procedure was launched. The following reactions were performed using reagents obtained from a Dual Luciferase Assay Kit (Promega). Cells were washed and lysed with the addition of 10 µl of passive lysis buffer (Promega). Following agitation (15 to 30 mins), luciferase activities of the plates were read in a dual-injector luminometer (BMG lumistar). For this purpose, 50 µl of luciferase assay reagent and 50 µl of 'Stop & Glo' buffer were injected sequentially to quantify the activities of both luciferases. Data obtained from the measurements were processed and analysed using suitable software. The mean Luciferase activity value obtained in wells A11 to H11 (Column 11, TGF-β only) was considered to represent 100% and values obtained in wells A12 to D12 (cells in

medium alone) gave a basal level (0%). For each of the compounds tested, a concentration response curve was constructed from which an $IC_{50}$ value can be determined graphically.

Assay 2 (Alk5 Fluorescence Polarization Assay)
Kinase inhibitor compounds, conjugated to fluorophores, can be used as fluorescent ligands to monitor ATP competitive binding of other compounds to a given kinase. The increase in depolarization of plane polarized light, caused by release of the bound ligand into solution, is measured as a polarization/anisotropy value. This protocol details the use of a rhodamine green-labeled ligand for assays using recombinant GST-ALK5 (residues 198-503).

Assay buffer components: 62.5 mM Hepes pH 7.5 (Sigma H-4034), 1 mM DTT (Sigma D-0632), 12.5 mM $MgCl_2$ (Sigma M-9272), 1.25 mM CHAPS (Sigma C-3023)

Protocol: Solid compound stocks were dissolved in 100% DMSO to 1 mM and transferred into column 1, rows A-H of a 96-well, U bottom, polypropylene plate (Costar #3365) to make a compound plate. The compounds were serially diluted (3-fold in 100% DMSO) across the plate to column 11 to yield 11 concentrations for each test compound. Column 12 contains only DMSO. A Rapidplate™-96 was used to transfer 1 µl of sample from each well into a 96-well, black, U bottom, non-treated plate (Costar #3792) to create an assay plate. These assay plates were ready for adding reagents.

ALK5 was added to assay buffer containing the above components and 1 nM of the rhodamine green-labelled ligand so that the final ALK5 concentration was 10 nM based on active site titration of the enzyme. 39 µl of the enzyme/ligand reagent was added to each well of the previously prepared assay plates. A control compound(1 µl) was added to column 12, rows E-H for the low control values. The plates were read immediately on a LJL Acquest fluorescence reader (Molecular Devices, serial number AQ1048) with excitation, emission, and dichroic filters of 485nm, 530 nm, and 505 nm, respectively. The fluorescence polarization for each well was calculated by the Acquest reader and then imported into curve fitting software for construction of concentration response curves. The normalized response was determined relative to the high controls (1 µl DMSO in column 12, rows A-D) and the

low controls (1 µl of control compound in column 12, rows E-H). An $IC_{50}$ value was then calculated for each compound.

Using the above assays all Examples of the invention showed ALK5 receptor modulator activity (having $IC_{50}$ values in the range of 1 to 200 nM) and TGF-β cellular activity (having $IC_{50}$ values in the range of 0.001 to 10µM).

2-(6-Methyl-pyridin-2-yl)-4-(pyridin-4-yl-amino)-quinazoline (Example 1) showed an ALK5 receptor modulator activity of 16 nM and TGF-β cellular activity of 82 nM.

2-(6-Methyl-pyridin-2-yl)-4-(pyrimidin-4-ylamino)-thieno[3,2-d]pyrimidine (Example 13) showed an ALK5 receptor modulator activity of 15 nM and TGF-β cellular activity of 23 nM.

Photoaging

The role of ALK5 inhibitors for treating photoaging was demonstrated by the following experiments.

Experiment 1 (see Figure 1)

Mice (BALB/c mice, 5 per group) were irradiated with ultra violet (UV) radiation for 30 minutes. The mouse ears were collected at 2h, 4h, 24h, 48h, 72h, and 96h post UV) together with the ears from untreated mice. RNA was extracted from the ears by acidified phenol chlorophorm extraction with ethanol precipitation. RNA levels were analyzed by quantitative reverse transciption polymerase chain reaction (TaqMan RT-PCR, Applied Biosystems, Foster City, CA). The results are shown in Figure 1 and show an increase in the levels of TGF-β 1 mRNA compared with normal ears. The maximum level was reached 24 hours after irradiation.

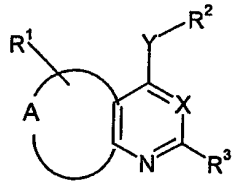
Experiment 2 (Figure 2)

This experiment looked at the long term effects (i.e. 5 and 6 days post irradiation). The mRNA species examined were TGF-β, plasminogen activator inhibitor-1 (PAI-1), fibronectin, the TGFbeta type I recetor (ALK5) and the TGF-beta type II receptor. The results are shown in Figure 2 and show a transient increase in TGF-β mRNA within 72 hours followed by a longer term increase in PAI-1 mRNA. This suggests that UV

irratiation activates the TGF-β system and may contribute to disordered collagen deposition and aging of the skin.

Claims

1. A compound of formula (I), a pharmaceutically acceptable salt, solvate or derivative thereof,



(I)

wherein

X is N or CH;

Y is –NH- or –N($C_{1-6}$alkyl)- or -NH-$CH_2$-;

A is a fused 5-, 6- or 7-membered carbocyclic or heterocyclic ring in which one or more of the carbon atoms is optionally replaced by a heteroatom independently selected from N, O and S, and wherein the carbocyclic or heterocyclic ring may be substituted by one or more $R^1$ groups;

$R^1$ is hydrogen, halo, nitro, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, -$CONR^4R^5$, -$O(CH_2)_nNR^4R^5$, -$(CH_2)_nNR^4R^5$ or -$NR^4R^5$, wherein $R^4$ is H or $C_{1-4}$ alkyl, and $R^5$ is $C_{1-4}$alkyl; or $R^4$ and $R^5$ together with the atom to which they are attached form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S and O;

$R^2$ is pyridinyl, pyrimidinyl, indazolyl, dihydroisoindolyl, benzisoxazolyl, oxazolyl, imidazolyl, oxadiazolyl or thiazolyl, each of which may be substituted by one or more $R^6$ groups;

$R^3$ is pyridin-2-yl, $C_{1-6}$alkylpyridin-2-yl, $C_{1-6}$alkylpyrrol-2-yl or $C_{1-6}$alkylthiazol-2-yl;

$R^6$ is hydrogen, halo, $C_{1-6}$alkyl, $C_{1-6}$alkoxy, -$O(CH_2)_nNR^7R^8$, -$O(CH_2)_n$-$OR^7$, -$NR^7R^8$, -$(CH_2)_nNR^7R^8$, -$CH_2OR^7$ , –$COOR^7$, –$CONR^7R^8$, -$CH_2SO_2NR^7R^8$, -$SO_2NR^7R^8$ or phenyl optionally substituted by one or more groups independently selected from the list: –$OCF_3$, halo,

$C_{1-6}$alkoxy, $-CONR^7R^8$, $-SO_2R^7$, $-O(CH_2)_nNR^7R^8$, $-(CH_2)_nNR^7R^8$ and $-NR^7R^8$;

$R^7$ and $R^8$, which may be the same or different, are H or $C_{1-6}$alkyl; or $R^7$ and $R^8$, together with the atom to which they are attached, form a 3-, 4-, 5-, 6- or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S and O; and

n is an integer value from 1 to 6.

2. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to claim 1 wherein X is N.

3. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to claim any preceding claim wherein Y is –NH-.

4. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to any preceding claim wherein ring A is thiophene, benzene, furan, pyridine, pyrazole or imidazole, each of which may be substituted by one or more $R^1$ groups.

5. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to claim 4 wherein ring A is thiophene or benzene either of which may be substituted by one $R^1$ group.

6. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to any preceding claim wherein $R^1$ is hydrogen, halo or $C_{1-6}$alkyl.

7. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to any preceding claim wherein $R^2$ is pyridinyl, pyrimidinyl or indazolyl, each of which may be substituted by one or more $R^6$ groups.

8. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to claim 7 wherein $R^2$ is pyridinyl, halopyridinyl, $C_{1-6}$alkylpyridinyl, pyrimidinyl or indazolyl.

9. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to any preceding claim wherein $R^3$ is pyridin-2-yl, 6-methylpyridin-2-yl or 4-methylthiazol-2-yl.

10. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, according to any preceding claim wherein $R^6$ is hydrogen or $C_{1-6}$alkyl.

11. A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, acccording to claim 1wherein

X is N;

Y is –NH-;

A is thiophene, benzene, furan, pyridine, pyrazole or imidazole, each of which may be optionally substituted by one of more $R^1$ groups;

$R^1$ is hydrogen, halo or $C_{1-6}$alkyl;

$R^2$ is pyridinyl, pyrimidinyl or indazolyl, each of which may be substituted by one or more $R^6$ groups;

$R^3$ is pyridin-2-yl, 6-methylpyridin-2-yl or 4-methylthiazol-2-yl; and

$R^6$ is hydrogen or $C_{1-6}$alkyl.

12. A compound according to claim 1 wherein the compound is:

2-(pyridin-2-yl)-4-(pyridin-4-ylamino)-thieno[3,2-d]pyrimidine;

2-(4-methyl-thiazol-2-yl)-4-(pyridin-4-ylamino)-quinazoline;

2-(6-methyl-pyridin-2-yl)-4-(pyrimidin-4-ylamino)-thieno[3,2-d]pyrimidine;

2-(6-methyl-pyridin-2-yl)-4-(1H-indazol-5-ylamino)-quinazoline;

2-(6-methyl-pyridin-2-yl)-4-(pyridin-4-ylamino)-quinazoline;

2-(6-methyl-pyridin-2-yl)-4-(pyridin-4-ylamino)-quinoline;

2-(4-methyl-thiazol-2-yl)-4-(pyridin-4-ylamino)-thieno[3,2-d]pyrimidine;

5-methyl-2-(pyridin-2-yl)-4-(pyridin-4-ylamino)-thieno[2,3-d]pyrimidine;

6-chloro-2-(6-methyl-pyridin-2-yl)-4-(1H-indazol-5-ylamino)-quinazoline; or

2-(6-methyl-pyridin-2-yl)-4-(pyrimidin-4-ylamino)-quinazoline

or pharmaceutically acceptable salts, solvates or derivatives thereof.

13 A pharmaceutical composition comprising a compound, a pharmaceutically acceptable salt, solvate or derivative thereof, defined in any preceding claim and a pharmaceutically acceptable carrier or diluent.

14 The use of a compound, a pharmaceutically acceptable salt, solvate or derivative thereof, defined in any one of claims 1 to 12 in the manufacture of a medicament for the treatment or prophylaxis of a disorder mediated by the ALK5 receptor in mammals.

15 The use according to claim 14 wherein the disorder is selected from chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers, ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to lung fibrosis, kidney fibrosis, liver fibrosis [for example, hepatitis B virus (HBV), hepatitis C virus (HCV)], alcohol induced hepatitis, retroperitoneal fibrosis, mesenteric fibrosis, haemochromatosis and primary biliary cirrhosis, endometriosis, keloids and restenosis.

16 The use according to claim 15 wherein the disorder is kidney fibrosis.

17 A compound, a pharmaceutically acceptable salt, solvate or derivative thereof, defined in any one of claims 1 to 12 for use as a medicament.

Figure 1

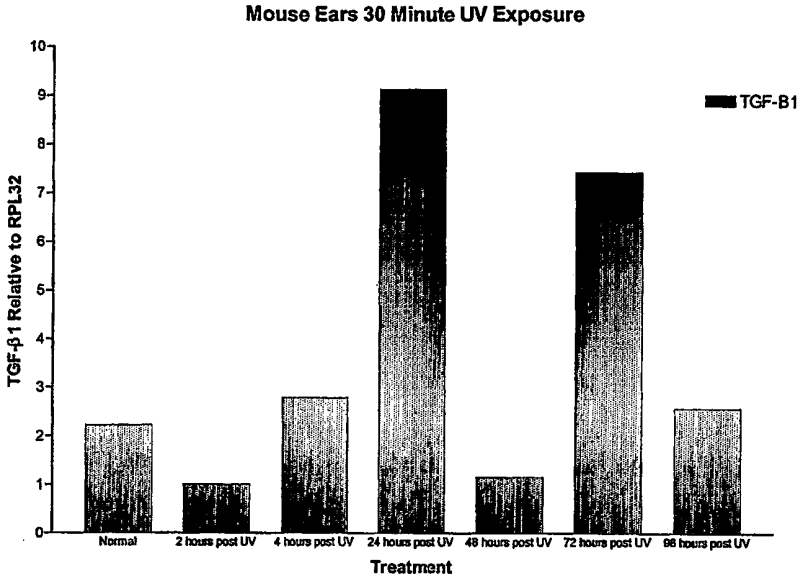
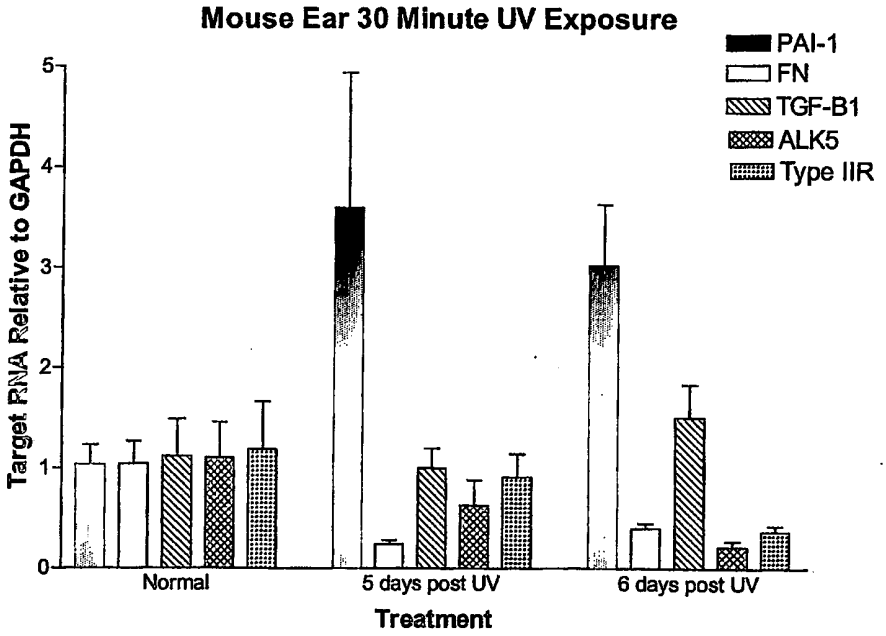



Figure 2



# INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/000650

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D495/04 C07D417/14 C07D401/14 A61K31/519 A61K31/517
A61K31/4709 A61P13/12 //(C07D495/04,333:00,239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 6 476 031 B1 (CHAKRAVARTY S ET AL)<br>5 November 2002 (2002-11-05)<br>cited in the application<br>the whole document, particularly table 2,<br>compound 52 | 1-17 |
| A | WO 02/062794 A (GLAXO GROUP LIMITED)<br>15 August 2002 (2002-08-15)<br>the whole document | 1,14 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 April 2004 | 29/04/2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Allard, M |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/000650

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 6476031 B1 | 05-11-2002 | US 6184226 B1 | 06-02-2001 |
| | | US 6277989 B1 | 21-08-2001 |
| | | US 2003069248 A1 | 10-04-2003 |
| | | US 2002161010 A1 | 31-10-2002 |
| | | AU 6241399 A | 21-03-2000 |
| | | BR 9913648 A | 02-01-2002 |
| | | CA 2342250 A1 | 09-03-2000 |
| | | CN 1333757 T | 30-01-2002 |
| | | EP 1107959 A2 | 20-06-2001 |
| | | JP 2002523502 T | 30-07-2002 |
| | | WO 0012497 A2 | 09-03-2000 |
| WO 02062794 A | 15-08-2002 | WO 02062794 A2 | 15-08-2002 |
| | | EP 1358187 A2 | 05-11-2003 |
| | | US 2004063949 A1 | 01-04-2004 |